PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 August 1999 (09.08.99)	
International application No. PCT/EP98/08522	**Applicant's or agent's file reference** DIR0550
International filing date (day/month/year) 17 December 1998 (17.12.98)	**Priority date** (day/month/year) 24 December 1997 (24.12.97)
Applicant BRANDS, Rudi	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

17 June 1999 (17.06.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Jean-Marie McAdams

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
DIR0550		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 98/ 08522	17/12/1998	24/12/1997
Applicant		
DUPHAR INTERNATIONAL RESEARCH B.V. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/08522

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N5/00 C12N7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 10564 A (US ARMY ;CELLCO (US)) 25 June 1992 see the whole document ---	1-6
X	EP 0 417 531 A (BAYER AG) 20 March 1991 see the whole document ---	1-6
X	WO 89 08701 A (INST ANGEWANDTE BIOTECHNOLOGIE) 21 September 1989 see the whole document -----	1-6

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 April 1999	10/05/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hillenbrand, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

[redacted]ation on patent family members

[redacted]ternational Application No
[redacted]T/EP 98/08522

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9210564 A	25-06-1992	AU 650711 B	30-06-1994
		AU 9124691 A	08-07-1992
		CA 2098510 A	14-06-1992
		EP 0564539 A	13-10-1993
		JP 6500927 T	27-01-1994
EP 0417531 A	20-03-1991	DE 3930140 A	21-03-1991
		DD 297663 A	16-01-1992
		JP 3098578 A	24-04-1991
WO 8908701 A	21-09-1989	DE 3833925 A	21-09-1989
		EP 0357738 A	14-03-1990
		JP 2503865 T	15-11-1990

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
DIR 0550		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/08522	17/12/1998	24/12/1997
International Patent Classification (IPC) or national classification and IPC		
C12N5/00		
Applicant		
DUPHAR INTERNATIONAL RESEARCH B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/06/1999	15.03.2000
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Hillenbrand, G Telephone No. +49 89 2399 8428

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/08522

I. Basis of th report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-10 as originally filed

Claims, No.:

1-6 as originally filed

Drawings, sheets:

1 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-6
102	No:	Claims	
Inventive step (IS)	Yes:	Claims	
103	No:	Claims	1-6
Industrial applicability (IA)	Yes:	Claims	1-6
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/EP98/08522

The examination is being carried out on the following application documents:

Text for the Contracting States:
AT BE CH DE DK ES FI FR GB GR IT IE LI LU MC NL PT SE

Description, pages:

1-10 as originally filed

Claims, No.:

1-6 as originally filed

Drawings, sheets:

1 as originally filed

Relevant documents cited

D1: WO 92 10564 A (US ARMY ;CELLCO (US)) 25 June 1992

Paragraph V (Reasoned statement) :

Novelty (Article 33.2 PCT):

Having regard to the documents cited in the International Search Report the claimed matter is considered to be novel.

Inventive step (Article 33.3 PCT):

D1 describes the production of high titers of recombinant viral vectors and transduced target cells in a sustained and continuous process (see Fig. 2). The difference between **D1** and the claimed matter appears to be the fact that the applicant claims a repeated discontinuous process. In view of **D1** the IPEA considers carrying out the process described in **D1** in a discontinuous manner by (a) using part of the cells of the preproduction batch for at least one production batch, and (b) using

the remaining part of the cells as a seed for the preparation of at least one subsequent preproduction batch as an obvious modification of the process described already in the cited prior art which does not require any inventive activity. This applies also to the use of a specific type of anchorage-dependent cells (see Claims 4-5). The fact that as a result of the claimed process it is possible to produce the product with cells at any passage number once a specific validation has been performed was also not surprising for the skilled person but the logical result of such a discontinues process.

Paragraph VIII (Certain observations on the international application):

The subject-matter of Claim 1 is too broadly and imprecisely drafted and thus does not comply with the requirements of Article 6 PCT. In Claims 4-5 the term "anchorage dependent" should be more clearly defined.

Finally, the attention of the applicant is directed to the fact that at present it appears that the claimed matter is not sufficiently clearly delimited from the teachings of US-A-5,017,490 and US-A- 4,664,912 (see page 1 of the description).

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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 5/00, 7/00

A1

(11) International Publication Number: WO 99/33955

(43) International Publication Date: 8 July 1999 (08.07.99)

(21) International Application Number: PCT/EP98/08522

(22) International Filing Date: 17 December 1998 (17.12.98)

(30) Priority Data:
97204110.7 24 December 1997 (24.12.97) EP

(71) Applicant *(for all designated States except US)*: DUPHAR INTERNATIONAL RESEARCH B.V. [NL/NL]; C. J. van Houtenlaan 36, NL–1381 CP Weesp (NL).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: BRANDS, Rudi [NL/NL]; Duphar International Research B.V., C. J. van Houtenlaan 36, NL–1381 CP Weesp (NL).

(74) Agent: BREEPOEL, Peter, Maria; Octrooibureau Zoan B.V., C. J. van Houtenlaan 36, NL–1381 CP Weesp (NL).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PREPARATION OF CELLS FOR PRODUCTION OF BIOLOGICALS

(57) Abstract

The present invention relates to a method for the preparation of cells for use in the production of biologicals, by culturing cells up to a desired cell volume of a preproduction batch, where after in a repeated discontinous process: a) part of the cells of the preproduction batch is used for the preparation of at least one production batch, and b) the remaining part of the cells of the preproduction batch is used as a seed for the preparation of at least one subsequent preproduction batch.

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Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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Preparation of cells for production of biologicals

The present invention is concerned with a method for the preparation of cells for use in the production of biologicals.

For the production of biologicals on e.g. cell lines, the preparation of large amounts of cells using an scaling up procedure in bioreactors will be necessary.

The US patent No. 5,017,490 discloses such a scaling up procedure which provides in particular the advantage of a low risk of transfer contamination. This method is, however, not suited for anchorage dependent cells (hence, not for cells which only grow if fixed to a substrate) or cells embedded in a substrate (e.g. in porous carriers).

The US patent No. 4,644,912 discloses a method for the preparation of anchorage-dependent cells for the production of biologicals (i.e. viruses) starting with a cell working seed, and with subsequent passages effected in increasing consecutive volumes of 1 litre, 5 litre, 25 litre, 150 litre bioreactors, and finally either in a 1000 litre bioreactor or in a multiplicity of 150 litre bioreactors. In between any of these passage steps the cells were released from their carriers with a dilute protease solution. In the final passage the inoculation by the virus was effected.

Assuming average cell cycle times of about 20-24 hours the passage intervals may be about every 3-5 day. Therefore, in order to expand the cells to sufficient large cultures from a MWCS[1] the total scaling up procedure may take several weeks, depending on the final bioreactor volume.

In the above methods for preparation of cells each of the ultimate production batches has to be prepared from the MWCS. For the production of vast amounts of biologicals it will be necessary to utilise several parallel culturing lines up to the largest vessel volumes. Such preparation procedure, hence, is very time consuming and necessitates the operation of a very considerable number of bioreactors for the preparation of the cells as well as for the production of the biologicals.

It is an object of the present invention to provide a much faster through-put in preparation of cells for the production of biologicals.

[1] MWCS = manufacturer's working cell bank

Accordingly, the present invention relates to a method for the preparation of cells for use in the production of biologicals, by culturing cells up till a desired cell volume of a preproduction batch, where after in a repeated discontinuous process:

a) part of the cells of the preproduction batch is used for the preparation of at least one production batch, and

b) the remaining part of the cells of the preproduction batch is used as a seed for the preparation of at least one subsequent preproduction batch.

More in particular, the present invention relates to a method for the preparation of cells for use in the production of biologicals, by culturing cells up till a desired cell volume of a preproduction batch, where after in a repeated discontinuous process:

a) part of the cells of the preproduction batch is transferred to be used for the preparation of at least one production batch, and

b) the remaining part of the cells of the preproduction batch is transferred to be used as a seed for the preparation of at least one subsequent preproduction batch.

In a preferred embodiment of the present invention the first preproduction batch is prepared from a working seed stock by at least one passage step.

In a further preferred embodiment of the present invention the cells which are prepared are anchorage-dependent. In the latter case it will generally be necessary that the cells are grown on a substrate. It will then be advisable during the repeated process each time when part of a batch is used for the preparation of a new batch to add an additional amount of substrate. In a preferred embodiment, each time prior to the addition of substrate at least part of the cells are first released from their original substrate

As used herein the expression "production batch" means a culture of cells which is employed for the production of biologicals.

As used herein the expression "preproduction batch" means a culture of cells which is used in the process according to the present invention for the preparation of at least one production batch (as defined above) and one subsequent preproduction batch.

As used herein the expression "biological" means any substance or organism which can be produced from a cell culture. Examples of "biologicals" are viruses and proteins such as enzymes.

As used herein the expression "working seed stock" means an amount of a certain type of cells of defined ancestry stored to be used as a seed from which all cultures of the same type of cells are derived.

As used herein the expression "anchorage-dependent cells" means cells which for their proper growing and/or propagation need to be attached to a substrate as defined herein.

As used herein the expression "substrate" means any particulate matter useful for the attachment of cells.

As used herein the expression "passage step" means a sequence of activities in the propagation and production of cells comprising at least the transfer of a suitable amount of cells and of a suitable amount of culturing medium into a production vessel, the incubation of the vessel at conditions suitable for the growing and propagation of the cells during a time sufficient for effective growing and propagation of the cells. Optionally a passage step may comprise separation of the cells from the culture medium and/or from the substrate after a time sufficient for effective growing and propagation of the cells.

It will be clear to the man skilled in the art that the method according to the present invention differs essentially from methods known in the art wherein cells are produced in a continuous process rather than the present discontinuous process. According to the patent publications EP0417531 and WO89/08701 continuous culture systems can be employed for the production of viruses as well. Firstly cells are grown in a first bioreactor, and after a certain cell density is reached cells are fed continuously from said first bioreactor into a second bioreactor. In this second bioreactor viruses are grown on the cells and subsequently these viruses are withdrawn continuously from this second bioreactor.

The basic method of working according to the present invention is to use a mother bioreactor from which the production bioreactor(s) is (are) fed with cells. When the cells are anchorage dependent, after each passage step cells preferably need to be detached from their substrates.
A trypsinisation procedure on large bioreactors has been developed for this purpose.
The production cells are defined up to a specific and characterised passage number for a so-call d ECB[2]. The method described allows high through-put production since the up scaling

[2] ECB = Extended Cell Bank

route from WCS to production cells can be very much shortened and much less bioreactors are needed since parallel production lines are not needed anymore.

Various embodiments of the present invention are depicted in Figure 1.

In a preferred embodiment cells are expanded from one ampoule of a MWCS up to the level of the first preproduction batch through one or more passage steps. The size of the bioreactor used for such a preproduction batch can range from several litres working volume to several hundreds of litres. Next, a part e.g. 10-20 % of the cells thus expanded (e.g. passage X) are used to repopulate a bioreactor for the production of a subsequent preproduction batch (being passage number X+1), whereas the bulk of the cells is transferred (passage X or X+1) to a larger bioreactor size in order to start production directly or to first populate it, and subsequently start production.

In classical serial production lines the number of doubling of the cells derived from the MWCS at the moment of harvest is known up front within certain limits. A maximum allowable generation number is set to the production system at the onset.

In the method according to the present invention the maximum number of cell passages can be defined by ECB. Production passage number (the number of cell passages used prior to production of the biological product), hence, is irrelevant within the limits set by ECB. As a consequence, such maximum number of passages is to be obeyed in view of regulatory restrictions. As a result the particular batch of produces biologicals is the end product of one direct scaling up route.

In order to verify whether the specifications of the cells at the stage of ECB in production are similar to the MCB[3] one need to perform specific validation for this purpose with respect to growth characteristics, freedom of adventitious, extraneous and endogenous agents at the different stages, karyology iso-enzyme analysis and so on. Once such ECB is fully characterised one may allow to produce the product with cells at any passage number between MCB and ECB, since it may be assumed that cells have not changed in between in their specs. As a result tests on the MWCS therefore can be limited to sterility testing. This is a particular advantage of the method according to the present invention

[3] MCB = Master Cell Bank

With the maximum passage number set one may use cells at any stage in between. From this in order to further minimise the time needed to expand the cells from the MWCS to production bioreactor it would be an advantage to enable bulk start-up of cells. This can be done for example in one of the following ways:

- Cells may be parked at a certain passage number during longer intervals at ambient temperature (17-32 °C) and be revitalised to log expansion growth by raising the temperature and changing the culture medium, or
- Cells may be frozen (Temp < -80°C) in bulk and be thawed prior to transfer them to a pre-set volume bioreactor, thereby reducing the needed up scaling route significantly.

The method according to the present invention can be carried out with animal cell cultures and more in particular with anchorage dependent cells. Suitable types of cells are e.g. hamster cells (CHO, BHK-1), monkey cells (Vero), bovine cells (MDBK), canine cells (MDCK), human cells (CaCo, A431) or chicken cells (CEF).

As a bioreactor according to the present inventions can be used a single unit of a plurality of units of e.g. stirred fermenters, fixed bed fermenters, fluidized bed fermenters, air lift fermenters, or a hollow fibre reactors.

Cells of the above times can and some even should be cultured when fixed to a solid support, like micro-carriers or macro-carriers in suspension, e.g. in a fixed bed, a fluidized bed or in suspension, or like hollow fibres. Cells can also be embedded into a carrier (e.g. porous carrier)

In the course of the method according to the present invention, in particular when using a solid support, cells are to be released from this solid support. This can be effected by any method useful for detaching of cells from a solid support. Advantageously, to this end use can be made of a proteolytic enzyme solution. Optionally, this enzymatic release step can be preceded by one or more pre-conditioning steps, e.g. by treatment with PBS and/or EDTA, in order to enhance the proteolytic efficiency, and/or in order to reduce the amount of proteolytic enzyme required.

EXAMPLE 1

Cell detachment and separation from carriers prior to transfer to next bioreactor

Anchorage dependent cells of a MDCK[4] cell line were cultured at 37 °C on Cytodex-3 micro carriers (Pharmacia, Uppsala, Sweden) (5 g of carriers/l) in a stirred bioreactor of 4 litre ("mother bioreactor"). The growth medium was EpiSerf (Life Technologies, Paisly, Scotland). Growth was continued till a maximum of $5x10^6$ cells/ml of culture.

The cells were detached from the carriers by trypsinisation in a Trypsin-EDTA solution (Life Technologies, Paisly, Scotland).

After settling of the carriers 80% of the detached cells were transferred to 3 other bioreactors of similar size. The latter "production" bioreactors all have carriers (cell substrate) added to them up front. Cells were allowed to repopulate the carriers and subsequently used for production in these production bioreactors.

The remainder of the cells in the "mother bioreactor" were allowed to repopulate the remaining Cytodex-3 carriers and were cultured to the desired cell density.

EXAMPLE 2

Cell detachment without separation from carriers prior to transfer to next bioreactor

The culturing of cells was carried out as described in Example 1, however after trypsinisation 80% of the detached cells including the carriers are transferred to the 3 production bioreactors. Additionally, suitable carriers were added to all bioreactors.

[4] MDCK = Madin Darby Canine Kidney (cell line)

EXAMPLE 3

Cell detachment without separation from carriers after transfer to next bioreactor

The culturing of cells was carried out as described in Example 1, however, 80 % of still adhered cells were transferred to a bioreactor of similar size which next was used directly for product generation.

The remaining cells on micro carriers in the mother fermenter were next detached by trypsinisation, where after new carriers were added and cells were allowed to repopulate the substrates.

EXAMPLE 4

Start-up from frozen bulk cells

In this experiment part of the culture was used to rebatch the mother fermenter and some daughter fermenters and part of the culture was used to freeze cells in bulk.

Frozen bulk cells (total $14.4X10^8$ cells) were inoculated in a start culture in a 3 litre mother fermenter containing 5 g Cytodex per litre and EpiSerf medium, and thereafter incubated at 37 °C. Residual cryo-preservatives were removed by a medium change on day 1.

At day 2 trypsinisation was carried out, 50% of the cells were bulk frozen and the remaining cells were inoculated to micro-carriers in a subsequent fermenter.

From Table 1 it can be deduced that the cells do continue to grow at a normal rate between day 2 and 3

On day 4 the content of the mother fermenter was trypsin-detached and rebatched onto new micro-carriers (10 g/l) in two other fermenters next to the mother fermenter.

At day 5 the plating efficiency turned out to be about 85%.

Tabl 1

day	3 litre mother ferm nt r c lls x 100.000/ml	3 litre ferm nt r cells x 100.000/ml	3 litre ferment r cells x 100.000/ml
0	NOD		
1	6.6		
2	14		
3	15.5		
4	30		
5	5.5	10	10
plating efficiency	85%	85%	85%

EXAMPLE 5

Transfer from small scale mother fermenter to large scale production fermenter

Cells were scaled up to a large scale in 65 litre and 550 litre fermenters (50 litre and 250 litre working volume, respectively) using a micro-carrier density of 5 g Cytodex per litre.

As can be seen from Table 2, 90% of the total of cells is transferred to the large scale fermenter from a 50 litre fermenter culture with 800.000 cells/ml of which 69% proved to be viable.

The same was found in the 50 litre mother fermenter; about 69% of the repropagating cells turned out to be viable.

The procedure was as follows:

On day 0, the carriers were allowed to settle in the 50 litre culture, where after the supernatant (culture medium) was removed and replaced by PBS. The content of the fermenter was agitated for 5-15 minutes. The supernatant was removed after resettling of the carriers. This step can be repeated if needed.

Next this step was repeated with PBS/EDTA (0.4 gram EDTA/litre PBS). Again the culture was agitated during 5-15 minutes, carriers were allowed to settle, the supernatant was removed, and the PBS/EDTA step was repeated until cells had become rounded and were ready to be trypsin-detached.

Then trypsin (0.025% final concentration) was added to the PBS/EDTA and incubated for 5-15 minutes. Next either the cell containing supernatant (after settling of now "nude" carriers) were transferred (as in xample 9) or the mixture of cells plus carriers were transferred (total 80 % of total mix).

After transfer of the cells to the 550 litre fermenter the remainder of the cells (hence, 10% of the viable cells) were allowed to repopulate the carriers still present in the fermenter after refilling the 50 l fermenter with culture medium.

About 70% of the cells proved to be viable

Table 2

day	50 litre culture cells x 100.000/ml	250 litre culture cells x 100.000/ml
0	8 (400 x 10^8 total cells)	1.1 (275 x 10^8 viable cells)
1		0.8
2		2.9
3		3.4
4		8.9
5		18.0

EXAMPLE 6

Analogous to Example 5, however, 80% of the culture of the carrier-bound cells were transferred from the mother bioreactor to the production bioreactor. Production was started after addition of virus.

The 20% of cells and carriers remaining in the mother bioreactor were trypsinized and detached and upon addition of new substrate into the mother bioreactor were allowed to repopulate the mother bioreactor while production is ongoing in the physically separated production bioreactor.

EXAMPLE 7

Large scale culture started from bulk frozen cells.

Bulk frozen cells were thawed and inoculated on a 10 litre (working volume) fermenter (Cytodex carrier density 5 g/l; culture medium EpiSerf) at an inoculation density of $1x10^6$ cells/ml. After attachment, the culture medium was replaced in order to remove residual cryo-protectants.

After day 1 the amount of viable cells attached to the carriers was $0.45x10^6$ cells/ml which from then on started growth. At a density of $2.8x10^6$ cells/ml the cells were detached from their

carriers by trypsinisation and 80 % was transferred to a 50 litre working volume fermenter (carriers 5 g/l).

As can be deduced from Table 3, at day 1 the amount of viable cells after bulk freezing of cells was about 45 %.

Of the total amount of transferred cells, the viability after trypsin detachment was 71.4%.

Table 3

day	cell density (x 10^5/l) in:	
	10 litre fermenter	50 litre fermenter
0	**1.0**	
1/2	**0.45**	
3/4	**1.3**	
5	**2.6**	
6	**2.8** (280 x 10^8 total)	
6	**0.6** (60 x 10^8 total)	**0.28** (140 x 10^8 total)
7		**0.4** (200 x 10^8 total)

Claims

1. Method for the preparation of cells for use in the production of biologicals, by culturing cells up till a desired cell volume of a preproduction batch, where after in a repeated discontinuous process:
   a) part of the cells of the preproduction batch is used for the preparation of at least one production batch, and
   b) the remaining part of the cells of the preproduction batch is used as a seed for the preparation of at least one subsequent preproduction batch.

2. Method according to claim 1 wherein in the repeated discontinuous process:
   a) part of the cells of the preproduction batch is transferred to be used for the preparation of at least one production batch, and
   b) the remaining part of the cells of the preproduction batch is transferred to be used as a seed for the preparation of at least one subsequent preproduction batch.

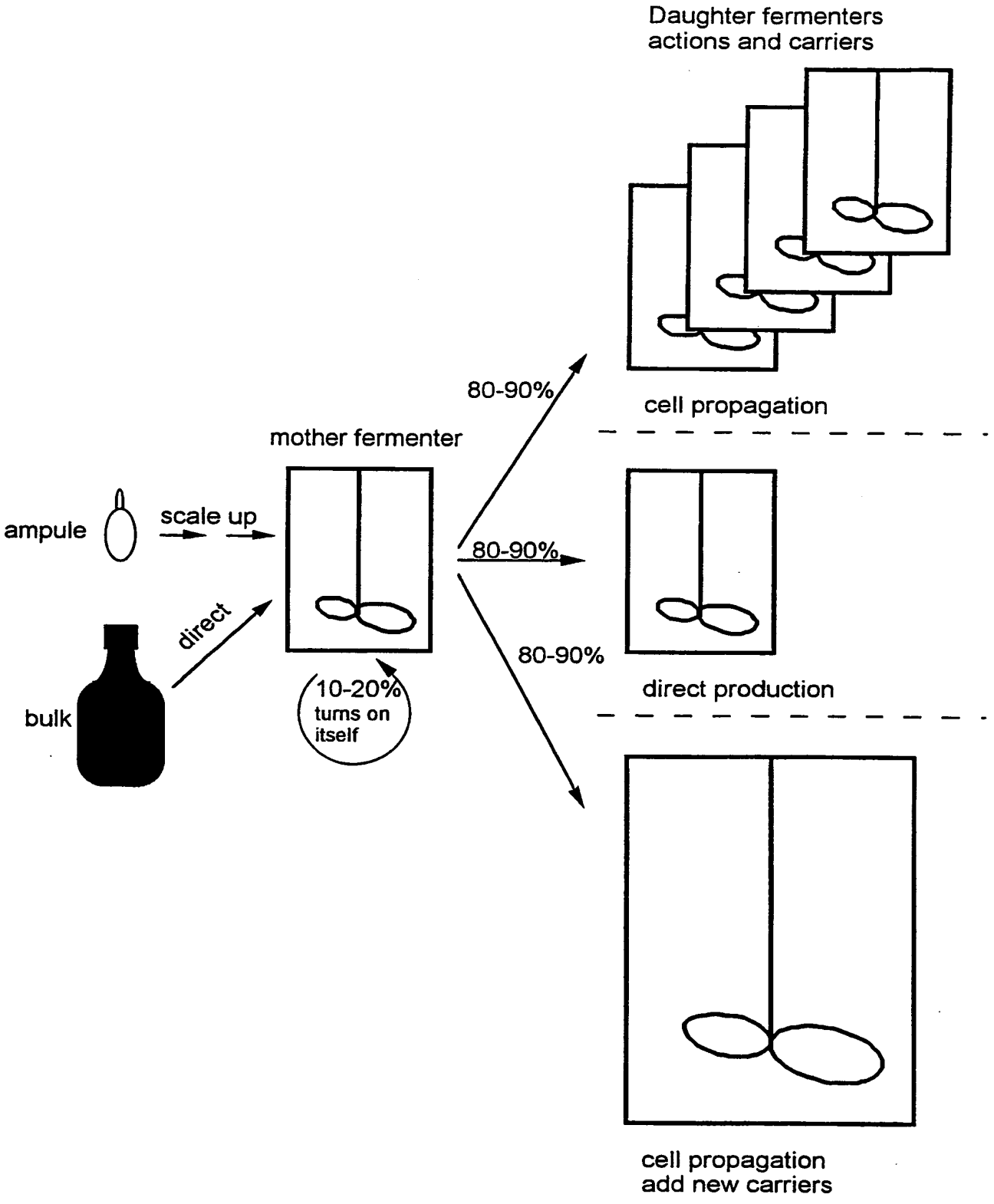
3. Method according to claim 1 or 2, characterised in that a first preproduction batch is prepared from a working seed stock by at least one passage step.

4. Method according to claim 1-3, characterised in that the cells are anchorage-dependent.

5. Method according to claim 2, characterised in that the cells are anchorage dependent, the cells are grown on a substrate, and prior to each transfer step the cells are released from their substrate.

6. Method according to claim 1-5, characterised in that the biological of interest is a virus.

FIGURE 1


Daughter fermenters
actions and carriers
80-90%
cell propagation
mother fermenter
ampule
scale up
80-90%
direct
80-90%
bulk
10-20%
turns on
itself
direct production
cell propagation
add new carriers

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N5/00 C12N7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 10564 A (US ARMY ;CELLCO (US)) 25 June 1992 see the whole document ---	1-6
X	EP 0 417 531 A (BAYER AG) 20 March 1991 see the whole document ---	1-6
X	WO 89 08701 A (INST ANGEWANDTE BIOTECHNOLOGIE) 21 September 1989 see the whole document -----	1-6

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
27 April 1999	10/05/1999

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